EXHIBIT C3

2dET Am Jrmec28
=> inhib titration

Cells trypsinized
Buffer A remade => Altered pH to 8
Buffer B remade => filtered
HPA: NE/DC (1.0/25)
Dilute
5mM DTT, Aprot, PMSF, 0.2mM vanadate

		2x OD_{405}	γ/γ	1γ	2.5γ
Cells killed 7pm (Harvest 12:30)	2N	.292	2.1	0.48	1.2
	2E	.353	2.8	0.36	0.9
	2E	.209	1.1	0.93	2.2
1mM ETD	2N	.235	1.4	0.7	1.75
	2E	.303	2.2	0.45	1.13
1mM DEVD	2N	.254	1.7	0.61	1.5
	2E	.231	2.0	0.51	1.27
5mM DEVD	2N	.220	2.0	0.51	1.28
	2E	.220	2.0	0.51	1.28
10mM DEVD	2N	.300	2.2	0.46	1.15
	2E	.262	1.7	0.57	1.43
1mM VETD	2N	.326	2.5	0.40	1.00
	2E	.255	1.7	0.60	1.50
5mM VETD	2N	.213	2.0	0.50	1.26
	2E	.277	1.9	0.52	1.31
10mM VETD	2N	.217	1.2	0.82	2.01
	2E	.256	1.7	0.60	1.49
10mM YVAD	2N	.288	2.0	0.49	1.22
	2E	.257	1.7	0.59	1.48
10mM LEHD	2N	.303	2.2	0.45	1.13
	2E	.288	2.0	0.49	1.22

21 hr/19

– Forgot to vortex after buffer A

*Sample 2N(8) got ice in 1.7

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			(3) 1		(3) 5		(3) 10	
(1)	2N	2E	2N	2E	2N	2E	2N	2E
	12	9 / 22	15	12.7	12.9	12.8	9.5	14.3
			(6) 1		(6) 5		(6) 10	
(2)	2N	2E	2N	2E	2N	2E	2N	2E
	12	9 / 22	10.1	15	12.6	13.1	20.4	9 / 14.9
			(8)		(1)			
(3)	2N	2E	2N	2E	2N	2E	2N	2E
	12	9 / 22	17.5	17.3	12.2	14.8	11.3	12.2

(4) 1γ βCAT — 2.5γ - PRO — 10γ βCAT

(M) 2N	2E	(M) 2N	2E	2N	2E
4.8	8.8 / 3.6 / 8.8	12	9 / 22	4.8+4.8	8.8+8.8

Note: loaded 1γ instead of 2.5γ of 2E for ECAD gels 4 prog
loaded 0.4γ instead of 1γ 2E for βCAT gel 1

Repeat

			(3) 1nM		(3) 5nM		(3) 10nM	
(1)	2N	2E	2N	2E	2N	2E	2N	2E
	4.8	8.8	6.1	5.1	5.1	5.1	4.6	5.7
			(6) 1nM		(6) 5nM		(6) 10nM	
(2)	2N	2E	2N	2E	2N	2E	2N	2E
	4.8	8.8	4.0	6.0	5.0	5.2	8.2	6.0
			(8)		(1)		(9)	
(3)	2N	2E	2N	2E	2N	2E	2N	2E
	4.8	8.8	7	4.5	4.9	5.9	4.5	4.9

(4) 1γ βCAT — 2.5γ - PRO — ~~10γ βCAT~~

(M) 2N	2E	(M) 2N	2E	(M) ~~2N~~	~~2E~~	~~(M) 2N~~	~~2E~~
4.8	8.8	12	22				

2N =
2E =

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Results:

~~Se~~ Gel (4) / Set 1

β Cat: see small amt. of cleaved product not seen in unstim. sample

p120ctn: definitely see shift, esp. in lower MW. regions

ECAD: no shift but see ~ 2X decrease

Gels (1), (2), (3), Set 2

- No signal or very faint ⇒ Redev. c̄ Pierce Reagent

Gel (1) - DEVD (3)

- control screwed up; ~~[illegible]~~
- 1nM (3), slight ↓ ECAD
- 5nM (3), ↓ in ECAD @ baseline, 3-4X ↓ in ECAD by ET-1
- 10nM (3), ECAD level down to ~~[illegible]~~ barely detectable

Gel (2) - VGTD (6)

- control screwed up
- (6) 1nM, 5nM: ECAD levels similar between stimulated and unstimulated samples
- 10nM ~~[illegible]~~ marked downreg of ~~ET~~ ECAD in ET-1 stimulated cells

Gel (3)

- control screwed up
- (8) 1nM: significant inhib of ↓ECAD
- (9) & 9nM: Marked downreg of ECAD by ET-1

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Concl:
- too little protein + to short Ab inc. time
- control screwed-up 2° to quantitation error
  ↳ - requantitate samps
    - load 25γ extract
    - 1°/45' (1°/2°) Ab incubation

- YVAD, LGHD @ 10nM ∅ effect on ↓ECAD by ETY
  ①
  ∴ Caspase 1, 4, 5, 9 not likely to be involved
  ↳ do one more trial of ①, ⑨ @ 1nM

- DEVD, IETD, and VEID interfere c̄ ↓ECAD by ETY.
  - have already ruled out Caspase 3, 7
  - have ruled in Caspase 8
  - need to ✓ Caspase 6, 10.